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ARTICLE

Quantification of Iprodione in Dry Basil Using Silica Gel Supported Titanium Dioxide

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Iprodione is an agricultural fungicide that is difficult to detect in foods by HPLC because it coelutes with natural compounds in the food. We previously showed that food matrix could be degraded with titanium dioxide powder (TP). Here we describe an improved method for detection of iprodione using silica gel supported titanium dioxide (SGT). To synthesize SGT, titania-sol was mixed with diethanolamine, 2-propanol, and titanium tetraisopropoxide. After titania-sol was infiltrated into the silica gel (particle diameter 4 mm), the mixture was dried and then heated. Crude basil extract containing iprodione was mixed with SGT in a quartz vial, and the vial was irradiated with a UV light to selectively decompose the matrix interfering with the iprodione determination. In HPLC chromatograms of the treated solution, the interference peak decreased 35 times faster with SGT than with TP. When SGT (11 g) was added to the extract (20 mL) of dry basil (2 g), black light irradiation for 30 min was enough to quantify iprodione. The recovery rate of iprodione was 99.1%. Thus, the photocatalytic cleanup method using SGT is effective for analyzing residual iprodione in dry basil.

KEYWORDS: Silica gel supported titanium dioxide; photocatalytic cleanup; basil; iprodione

INTRODUCTION

To determine residual agricultural chemicals such as iprodione in foods, agricultural chemicals are usually extracted into organic solvents and purified using cartridge columns packed with silica gel, magnesium silicate, amino propyl, or ion-exchange resin. The agricultural chemicals are then determined by gas chromatography (GC), GC-mass spectrometry (GC-MS), liquid chromatography−mass spectrometry (LC-MS), or high performance liquid chromatography (HPLC) (*1*, *2*).

Iprodione is an agricultural fungicide that can be toxic to humans at high concentrations. For some foods, such as dry fruits, vegetables, and processed foods, detecting iprodione can be difficult because it coelutes with natural compounds in the foods.

Previously, to avoid this problem, we developed a cleanup method that uses fine titanium dioxide powder (TP, 0.1−0.3 μm), which decomposes the food matrix faster than it decomposes iprodione (*3*). However, two problems with this method are that the TP must be removed from the test solution before HPLC analysis and decomposition of the matrix takes a long time (∼3 h) because the catalytic reaction of TP occurs only on the surface of the TP. To solve these problems, we synthesized a silica gel supported titanium dioxide (SGT) via photocatalysis. National Institute of Health Sciences, Japan, investigated the residual agricultural chemicals in the imported foods (*4*). As a result of the survey from 2005 to 2006, basil was found to be one of the highly contaminated foods with agricultural chemicals, and iprodione was a contaminating agricultural chemical with a high detection rate. We selected dry basil as a test food and iprodione as a target agricultural chemical. Using dry basil as a test food, we demonstrate that SGT is more effective for quantifying iprodione than TP.

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MATERIALS AND METHODS

Reagents. Iprodione, 3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide (analytical grade for residual pesticides), acetone (analysis grade for pesticides and polychlorinated biphenyl), acetonitrile (HPLC grade), 2-propanol, diethanolamine, and titanium tetraisopropoxide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Titanium dioxide (∼80% anatase form, particle size: 0.1−0.3 μm) was obtained from Kanto Chemical Co, Inc. (Tokyo, Japan). Silica gel (CARiACT Q-50, particle diameter: 4 mm) was manufactured by Fuji Silysia Chemical Ltd. (Aichi, Japan).

Instruments. The equipment used included a dry thermo unit (DTU-1B, Taitec Corporation, Saitama, Japan), a testing shaker (MMS-3011, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), black light lamps (FL10BLB-A, 10 W, peak wavelength: 352 nm, Toshiba Lighting and Technology Corporation, Tokyo, Japan), PTFE membrane filters (pore size: 0.45 μm, SUN-Sri, Rockwood, TN, USA), a centrifugal separator (KN-70, Kubota Corporation, Tokyo, Japan), and an electric muffle furnace (KM-1303, Advantec Toyo Kaisha, Ltd., Tokyo, Japan).

HPLC Analysis. The HPLC system used was an HP 1050 series (Hewlett-Packard Co., Palo Alto, CA) equipped with a UV−vis detector

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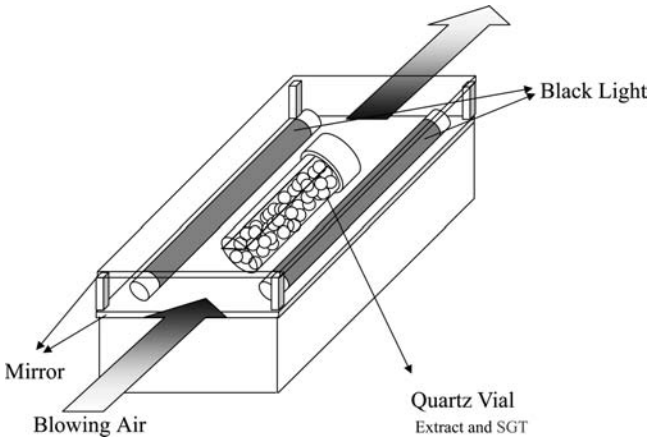

Figure 1. Illustration of the UV irradiation device. A quartz vial containing the mixture of dry basil extract and SGT was set between two black light sources. The vial and two black lights were sandwiched between two mirrors.

(UV-8020, Tosoh Corporation, Tokyo, Japan). A reversed-phase column (Shiseido CAPCELL PAK C18 UG120, 4.6 mm i.d. × 250 mm, 5 μm) and a guard column (CAPCELL C18 UG120 S-5, 4.0 mm × 10 mm) were kept at 25 °C in a column oven (L-7300, Hitachi Ltd., Tokyo, Japan). The mobile phase was 60% (v/v) acetonitrile containing 4.4 mM trifluoroacetic acid. The flow rate was 0.8 mL min^{-1}. After injecting the sample solution (10 μL), we monitored the absorbance of the eluent at 230 nm.

Measurement of Titanium. The concentration of titanium was measured using an energy dispersive X-ray analyzer (EDAX Japan KK., Tokyo, Japan) equipped with a scanning electron microscope (JSM-5400LV, JEOL Ltd., Tokyo, Japan). The acceleration voltage was 20 kV.

Preparation of SGT. SGT was prepared according to the method of Taoda et al. with several modifications (*5*, *6*). After mixing 10 mL of diethanolamine and 200 mL of 2-propanol, 30 mL of titanium tetraisopropoxide was added to the solution while stirring. The titania solution (240 mL) was mixed with silica gel (50 g), and the mixture was allowed to stand for 60 min at room temperature. SGT was washed with 2-propanol, dried at room temperature for 30 min, and then dried at 100 °C. SGT was activated by heating it at 600 °C in an electric muffle furnace for 120 min.

Preparation of Dry Basil Extract. Commercially available dry basil was purchased, crushed, and passed through a standard sieve (aperture: 425 μm). Three grams of the sieved, dry basil were weighed into a 50 mL glass centrifugation tube and mixed with 30 mL of acetone. The mixture was shaken for 10 min (amplitude: 30 mm; speed 150 min^{-1}) and then centrifuged at 3000 rpm for 5 min to obtain the crude extract.

Clean-up Using SGT. For UV irradiation, we used 10 W black light lamps (FL10BLB-A, Toshiba Lighting and Technology Corp., Tokyo, Japan) with a peak wavelength of 352 nm. Crude basil extract (20 mL) was placed in a 40 mL quartz vial, to which was added 11 g of SGT. The vial was capped and placed between the two black lights with mirrors above and below (**Figure 1**). By allowing air to flow through the system, the sample temperature was maintained between 23 and 25 °C. After 180 min of irradiation, 1 mL of the extract was dried under a nitrogen stream using a dry thermo unit at 35 °C. The precipitate was dissolved in 1 mL of acetonitrile. An aliquot of the solution was injected into the HPLC system.

Clean-up Using TP. The procedure was the same as that described for SGT except that the amount of TP used was 6 g. The vial was placed between the two black lights and shaken (amplitude: 30 mm; speed: 150 min^{-1}) for 180 min during irradiation. The sample was filtered using a disk filter (pore size: 0.45 μm), and 1 mL of the extract was dried under a nitrogen stream using a dry thermo unit at 35 °C. The precipitate was dissolved in 1 mL of acetonitrile, and the solution was centrifuged at 3000 rpm for 5 min. An aliquot of the supernatant was injected into the HPLC system (*3*).

RESULTS AND DISCUSSION

In our previous study, the substances that interfered with the HPLC measurement of iprodione were decomposed completely by TP acting as a photo-oxidation catalyst (*3*). However, titanium dioxide powder with a particle size of 0.1−0.3 μm must be removed by centrifugation. On the other hand, it was not necessary to centrifuge SGT to obtain the supernatant as described below.

Table 1. Distribution of Titanium in SGT (Particle Diameter, 4 mm; Pore Size 47.0 nm)

distance from surface (μm)	titanium concentration (g Ti/100 g silica gel)
0−700	1.61−3.33
700−1400	0.84−2.19
1400−1800	0−1.38
1800−2000	0
total (average)	2.07

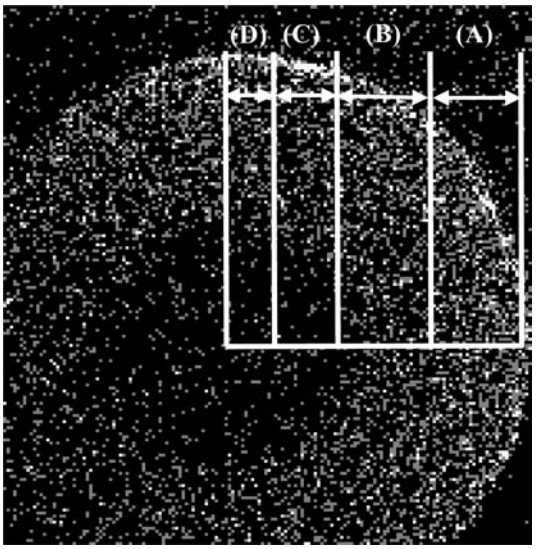

Figure 2. Typical elemental mapping of the titanium in cross-section of SGT (diameter 4 mm) by energy dispersive X-ray analyzer: (**A**) 700 μm from surface; (**B**) 700−1400 μm from surface; (**C**) 1400−1800 μm from surface; (**D**) 1800−2000 μm from surface (center).

Titanium Concentration in SGT. The amounts of oxygen, silicon, and titanium in the SGT were determined by energy dispersive X-ray analysis. The characteristics of the SGT including titanium concentrations are described in **Table 1**. The average concentration of titanium was 2.07% (3.45% as titanium dioxide) in SGT. The elemental mapping cross-section of SGT by the energy dispersive X-ray analyzer showed that titanium was found near the surface of SGT but not in the core (**Figure 2**).

Optimization of SGT Photocatalysis. Eleven grams of SGT was soaked completely in 20 mL of the basil extract. If more than 11 g of SGT were added, the surface of the solution was lower than the SGT layer. Thus, for 20 mL of basil extract, the optimum amount of added SGT was 11 g.

Shaking the sample solution during irradiation caused it to become a milky suspension of small crushed particles of SGT, so the sample was not shaken. After irradiation, the sample solution could be directly subjected to HPLC without centrifugation.

HPLC Analysis. Figure 3 shows a series of chromatograms after various treatments. **Figure 3A** shows the chromatogram of the standard iprodione solution (1.0 ppm in acetone) after irradiation for 180 min in the presence of 11 g of SGT. The basil extract (20 mL) was also irradiated for 180 min in the presence of 11 g of SGT. There is no peak at the retention time of iprodione (12.5 min) (**Figure 3B**). The basil extract (20 mL) was spiked with

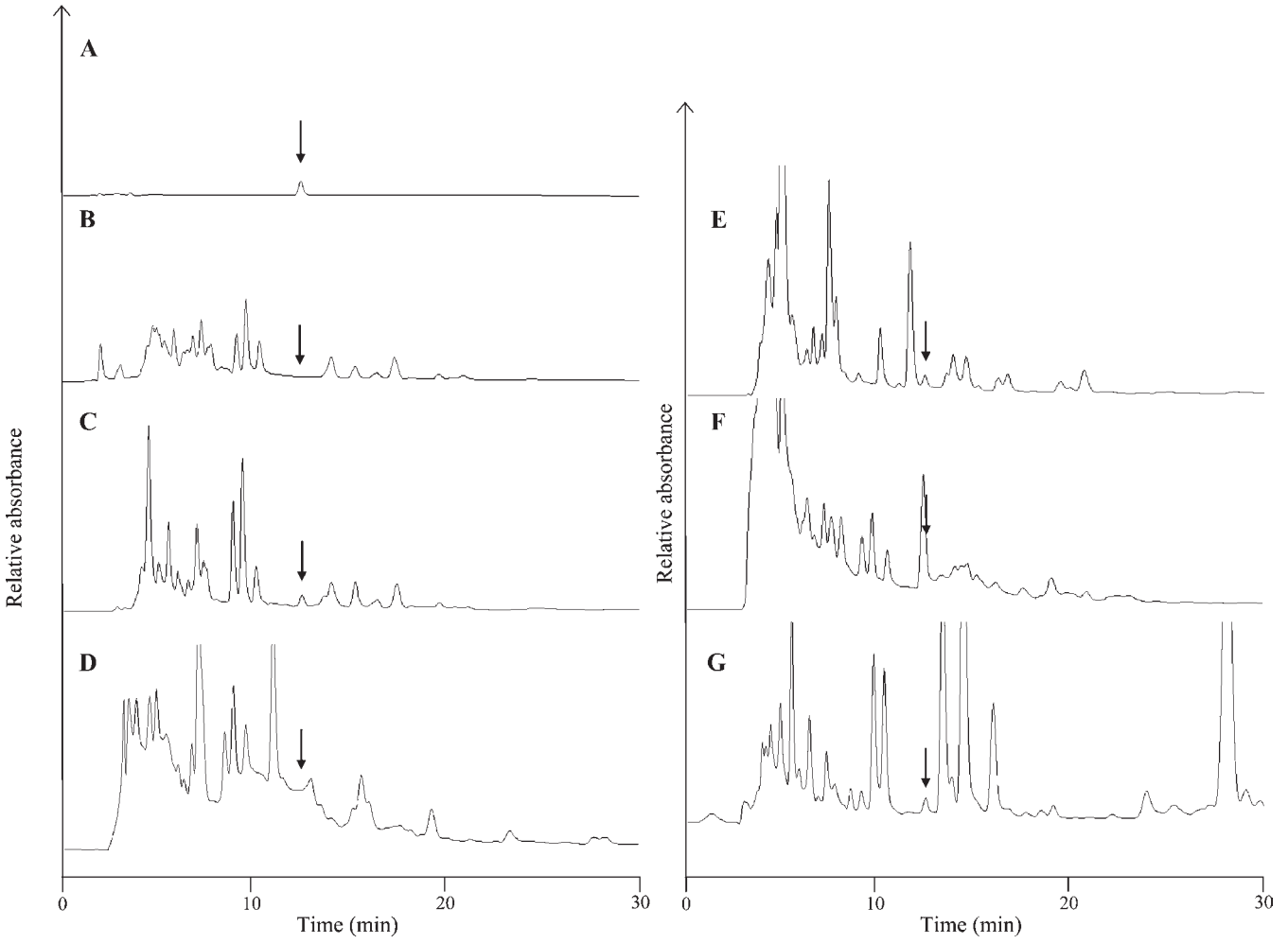

Figure 3. Typical HPLC chromatograms of iprodione, dry basil extract, and dry basil extract spiked with iprodione. (**A**) Iprodione (1 ppm) after 180 min irradiation in the presence of SGT (11 g). (**B**) Dry basil extract after 180 min irradiation in the presence of SGT (11 g). (**C**) Dry basil extract spiked with 1 ppm iprodione after 180 min irradiation in the presence of SGT (11 g). (**D**) Dry basil extract spiked with 1 ppm iprodione after 180 min irradiation in the presence of silica gel (11 g). (**E**) Dry basil extract spiked with 1 ppm iprodione after 180 min irradiation in the presence of TP (6 g). (**F**) Dry basil extract spiked with 1 ppm iprodione after 180 min irradiation in the presence of TP (0.38 g). (**G**) Dry basil extract spiked with 1 ppm iprodione after 30 min irradiation in the presence of SGT (11 g). The arrow shows the peak (or retention) time of iprodione.

iprodione (1 ppm) and irradiated for 180 min in the presence of 11 g of SGT. The iprodione peak could be quantified because there was no interference peak (**Figure 3C**). The limit of detection for iprodione was 0.5 ppm. However, after 180 min of irradiation in the presence of silica gel (CARiACT Q-50, 11 g), the background absorbance of the same sample around the retention time of iprodione was higher (**Figure 3D**). This result indicated that SGT effectively decomposed the components that interfered with the analysis of iprodione.

TP also decomposed the matrix of the basil extract (*3*). **Figure 3E** shows the chromatogram of the basil extract after irradiation for 180 min in the presence of 6 g of TP. When the added amount of TP was increased to reduce the irradiation time, an emulsion formed that was difficult to remove. The actual amount of titanium dioxide present in 11 g of SGT was 0.38 g, calculating from 3.45% titanium dioxide (2.07% titanium) in SGT as shown in **Table 1**. When 0.38 g of TP was added to the extract spiked with iprodione (1 ppm), the iprodione peak could not be resolved in this chromatogram (**Figure 3F**).

When 11 g of SGT was added to the extract, the required cleanup time (30 min) was less than 1/6 of that required in the presence of 6 g of TP, and 30 min of irradiation was sufficient for decomposition (**Figure 3G**). SGT required a shorter irradiation treatment time because the SGT is translucent in the extract, and therefore, UV light could reach the interior of the sample. In addition, SGT supports titanium not only on the surface of the silica gel but also in the fine pores.

Parts **C** and **E** of **Figure 3** show that background substances that interfered with the determination of iprodione were decomposed to the baseline, allowing iprodione to be quantified. The interfering peaks in **Figure 3C** were much less than those in **Figure 3E**, suggesting that the former conditions were much better. Additionally the recovery of iprodione did not change after the 180 min irradiation, suggesting that iprodione was not adsorbed to SGT.

Recovery of Iprodione and Decomposition of Interfering Substances. After the cleanup using SGT and TP, the height of the iprodione peak (at 12.5 min) was compared with the height of the interference peak (at 12.5 min) on the HPLC chromatogram (**Figure 4**). The recoveries of iprodione were 99.1 ± 1.0% using SGT and 102.6 ± 1.4% using TP. On the other hand, the height of the interference peak decreased to 2.1% when TP (6 g) was used. When SGT (11 g) was used, the peak height decreased to 2.5% after 30 min irradiation and decreased to 1.3% after 180 min irradiation. This result shows that the decrease of the interfering compounds was more rapid when using SGT in spite of the

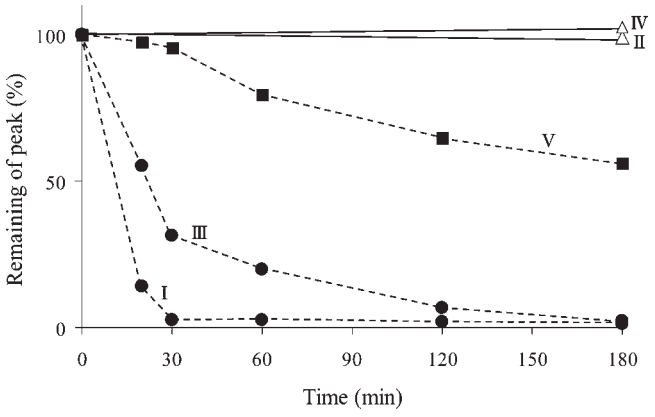

Figure 4. Time courses of interfering peak (12.5 min) and iprodione peak in the dry basil extract after dry basil extract spiked with iprodione (1 ppm) was irradiated in the presence of TP or SGT. (**I**) Interference peak in the presence of SGT (11 g). (**II**) Iprodione peak in the presence of SGT (11 g). (**III**) Interference peak in the presence of TP (6 g). (**IV**) Iprodione peak in the presence of TP (6 g). (**V**) Interference peak in the presence of TP (0.38 g).

smaller titanium dioxide content. The natural logarithms of the residual ratio of the interfering peak were obtained for SGT (11 g), TP (6 g), and TP (0.38 g). We fitted the plots using the least-squares method and calculated the decomposition rate constant as the slope of each plot. The decomposition rate constants (min^{-1}) for TP (0.38 g), TP (6 g), and SGT (11 g) were 0.0033, 0.0223, and 0.1154, respectively. The decomposition rate of SGT (11 g) was 35 times larger that that of TP (0.38 g) and 5.2 times larger that that of TP (6 g), indicating that SGT decomposes interfering substances in the basil extract more efficiently than TP.

Because this method does not require centrifugation or filtration after the irradiation, the SGT-treated sample can be directly injected into the HPLC system. We are now investigating whether the proposed method can be used to measure other agricultural chemicals in food samples.

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